Review

Coxiella burnetii: Host and bacterial responses to infection

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Abstract

Designation as a Category B biothreat agent has propelled *Coxiella burnetii* from a relatively obscure, underappreciated, "niche" microorganism on the periphery of bacteriology, to one of possibly great consequence if actually used in acts of bioterrorism. Advances in the study of this microorganism proceeded slowly, primarily because of the difficulty in studying this obligate intracellular pathogen that must be manipulated under biosafety level-3 conditions. The dogged determination of past and current *C. burnetii* researchers and the application of modern immunological and molecular techniques have more clearly defined the host and bacterial response to infection. This review is intended to provide a basic introduction to *C. burnetii* and Q fever, while emphasizing immunomodulatory properties, both positive and negative, of Q fever vaccines and *C. burnetii* infections.
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Contents

1. Introduction

1.1. History

Q fever was first observed in Australia in 1933 as a disease affecting slaughterhouse workers [1]. Symptoms of this previously uncharacterized disease were fever, headache, and malaise. Given the uncertain etiology, the disease was given the name Q fever (for query). However, the infectious agent

could not be isolated. At about the same time, ticks were being collected in Montana USA for investigations into Rocky Mountain spotted fever. Allowing the ticks to feed on guinea pigs resulted in a febrile response [2] and their inflammatory cells contained rickettsia-like microorganisms [3]. Therefore, in the US, an infectious microorganism was discovered, but the disease that it caused was unknown. However, in Australia a new disease was identified, but with an unknown etiology.

In a remarkable mix of serendipity and science, the Q fever agent and the Montana isolate were demonstrated to be identical since guinea pigs that were previously challenged

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14. ABSTRACT

Designation as a Category B biothreat agent has propelled Coxiella burnetii from a relatively obscure, underappreciated, niche microorganism on the periphery of bacteriology, to one of possibly great consequence if actually used in acts of bioterrorism. Advances in the study of this microorganism proceeded slowly, primarily because of the difficulty in studying this obligate intracellular pathogen that must be manipulated under biosafety level-3 conditions. The dogged determination of past and current C. burnetii researchers and the application of modern immunological and molecular techniques have more clearly defined the host and bacterial response to infection. This review is intended to provide a basic introduction to C. burnetii and Q fever, while emphasizing immunomodulatory properties, both positive and negative, of Q fever vaccines and C. burnetii infections.

15. SUBJECT TERMS
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with the Montana tick isolate were resistant to challenge with the Q fever isolate. Furthermore, serum from a patient previously infected with the Montana isolate was able to neutralize the infectivity of the Q fever agent [4]. Although initially named *Rickettsia diaporica* [5] and *Rickettsia burnetii* [6], the microorganism was given the name *Coxiella burnetii* in 1948 [7].

1.2. Life cycle

C. burnetii is an unusual microorganism, replicating only within the phagolysosomal vacuoles of host cells, primarily macrophages. During natural infections, the organism grows to high numbers in placental tissues of goats, sheep, and possibly cows [8,9]. In the laboratory, *C. burnetii* is routinely cultured in chicken embryo yolk sacs, in cell cultures, and can be recovered in large numbers from spleens of experimentally infected mice and guinea pigs [10].

The infectious particles, referred to as small cell variants (SCV), are responsible for the ability to survive extreme environmental conditions of desiccation, heat, sonication, and pressure [11,12]. In the host, the infecting SCV develop into large cell variants (LCV) that are metabolically active. The SCV and LCV are antigenically different [13]. Transition between SCV and LCV is accompanied by changes in the expression of surface proteins and does not involve classical phase variation, which refers to lipopolysaccharide (LPS) structure. Infectious particles have been referred to as "endospore-like", but this nomenclature is misleading because they are not structurally similar to *Bacillus* spores.

Like other Gram-negative microorganisms, *C. burnetii* possesses a LPS that is important in virulence and responsible for the antigenic phase variation, analogous to the smooth-rough LPS variation seen in enteric Gram-negative microorganisms [14,15]. Bacterial isolates from eukaryotic hosts have a phase I (smooth) LPS, which can protect the microorganism from microbicidal activities of the host. As isolates are passed in yolk sacs or other immune-incompetent hosts, the phase I LPS character of the bacterial population gradually changes, within approximately 20 yolk sac passages, to the phase II (rough) form that has chromosomal deletions in genes responsible for LPS O-side-chain biosynthesis [16,17]. Phase I microorganisms are virulent, while phase II microorganisms are avirulent in immune-competent hosts. Entry of phase II *C. burnetii* into host cells is via the phagolysosomal pathway and the CR3 receptor, whereas phase I cells bind monocytes via the leukocyte response integrin ($\alpha_v\beta_3$) and integrin-associated protein and are poorly internalized [18,19]. LPS is involved in the uptake of virulent, but not avirulent variants of *C. burnetii*, by a toll-like receptor (TLR) 4-dependent mechanism [20]. (However, TLR4 does not seem to be involved in activating macrophages to be microbicidal [20].) In addition to LPS, suggested virulence determinants include acid phosphatase, com1 protein, catalase, superoxide dismutase, and macrophage infectivity potentiator [21,22]. A type IV secretion system might also facilitate intracellular growth [23]. One strategy the microorganism uses to survive in the hostile environment of the phagolysosome might involve the production of oxygen scavengers [21]. An iron/manganese superoxide dismutase has been demonstrated and genetic sequencing has also revealed coding for a copper-zinc superoxide dismutase [24].

2. Pathogenicity/disease

Q fever is most commonly acquired by breathing infectious aerosols or dust contaminated with birth fluids of domestic ruminants [25]. The infectious dose for humans is estimated to be 10 microorganisms or fewer [26]. Of the variety of species that can be infected by *C. burnetii*, symptomatic disease seems to be most severe in humans. However, infection of ruminants is associated with abortion and decreased milk production [27,28]. Seropositivity to *C. burnetii* has also been associated with adverse human pregnancy outcomes, including neonatal death [29,30]. Q fever occurs world wide and the rates of seropositivity vary. Fourteen percent of human sera tested positive in Nova Scotia, Canada [31]. The pathogenesis of human infection is ill defined. Based on studies with animal models, after initial infection at the site of entry (usually the lungs), the microorganism is engulfed by resident macrophages and transported systemically, causing histopathological changes in the lungs, liver, and spleen [32]. After uptake by host cells, the acidic conditions within the phagolysosome allow the bacteria to grow [33]. Eventually, proliferation within the phagolysosome leads to rupture of the host cell and infection of a new population of host cells. In animal models, the spleen and liver and other tissues of the reticuloendothelial system appear to be most heavily infected, as is likely the case in human infection [34].

Although the majority of human *C. burnetii* infections are asymptomatic, overt cases of acute Q fever result in mild to moderate illness [35]. The incubation period can vary from a few days to several weeks and the severity of infection varies in direct proportion to the infectious dose in normal individuals [36,37]. There are no characteristic symptoms of Q fever, but fever, severe headache, and chills tend to be prevalent. Fever usually peaks at 40 °C and lasts for approximately 13 days [38]. Fatigue and sweats also frequently occur [39]. Pneumonia is a common clinical presentation [40]. Cough, nausea, vomiting, myalgia, arthralgia, chest pain, hepatitis, and occasionally, splenomegaly, osteomyelitis, and meningoencephalitis are symptoms that are also associated with acute Q fever [39,41]. Fatalities in cases of acute Q fever are rare, with fewer than 1% of cases resulting in death [1]. Children develop symptomatic disease less frequently than adults [42].

Although acute Q fever is a self-limited systemic illness, some patients develop a chronic debilitating disease. The risk of developing chronic Q fever after a case of acute Q fever ranges to 9% [43]. While chronic Q fever occurs

less frequently, it is more pathogenic than acute Q fever. Chronic Q fever can arise years after the initial presentation of acute disease. Patients with prior coronary disease or patients immunocompromised because of disease, such as AIDS, or therapy, such as immunosuppressive cancer therapy or anti-rejection therapy after organ transplant, are more at risk for developing chronic Q fever [44,45]. Endocarditis, primarily of the aortic and mitral valves [46], is the most common manifestation of chronic Q fever. Approximately 90% of Q fever endocarditis patients have pre-existing valvular heart disease [47]. Of acute Q fever patients with cardiac valve abnormalities, as many as one-third develop endocarditis [48].

Approximately 10% of people recovering from acute Q fever have fatigue lasting for longer than 6 months [49]. The cause of this syndrome, called Q fever fatigue syndrome, is thought to be high levels of cytokines, including interleukin (IL)-10, stimulated by persisting *C. burnetii* antigens [50]. Predisposing genetic factors resulting in immune hypersensitivity [51] that leads to higher levels of *C. burnetii* genomes in bone marrow and increased shedding into the peripheral blood are also thought to make patients more susceptible to developing Q fever endocarditis and chronic fatigue syndrome. For example, patients harboring the HLA DRB1*11 allele were more likely to develop Q fever fatigue syndrome. However, patients with chronic Q fever endocarditis were more likely to have differences in the IL-10 promoter microsatellites R and G and to have the TNF-alpha receptor II 196R polymorphism.

Whether *C. burnetii* strains associated with acute Q fever are genetically different from strains causing chronic disease is controversial. An immunodominant 28-kDa protein was found to be associated with strains causing acute Q fever, but not chronic disease [52]. In addition, allelic differences of several strains associated with chronic Q fever were not found associated with strains causing acute disease [53]. Therefore, it seems likely that the disease course in humans is related to the strain of the infecting microorganism. An unresolved question is whether humans ever completely clear the microorganism after infection. *Coxiella* DNA was found in the bone marrow of 88% of patients tested who had primary Q fever 12 years previously [49]. Chronic Q fever could result from sequestration of *C. burnetii* in the bone marrow with subsequent seeding of other tissues, such as the endocardium.

Control of infection is mediated by monocytes and macrophages that are activated by gamma interferon, resulting in the production of reactive nitrogen and oxygen intermediates, and leading to intracellular killing of the pathogen [54–56]. Although antibodies might facilitate bacterial entry into host cells and accelerate the development of immunity, passive transfer of antibodies, before or after challenge, did not control infection [57,58]. Other evidence that antibodies are not a primary mechanism of resistance against *C. burnetii* involves mice unable to express CD28, a molecule found on NK cells and activated T cells. Although these mice produced less IL-10 and fewer antibodies to *C. burnetii* than normal mice, they were more resistant to infec-

tion, having a lower burden of *C. burnetii* in spleens and livers after infection. However, their ability to produce granulomas and inflammatory cytokines was unimpaired [59]. These data also imply that by inducing IL-10, *C. burnetii* inhibits Th1 immune responses and the ability of the host to control infection.

The patient's antibody response to *C. burnetii* infection, together with nonspecific clinical signs and a history of possible exposure, can be used as evidence of recent infection and lead to therapeutic intervention. Levels of antibody responses directed against killed phase I and phase II cellular diagnostic antigens provide serological evidence that can support a clinical diagnosis of acute Q fever [60,61]. Determining relative titers of antibodies to phase I and phase II *C. burnetii* can also help distinguish acute from chronic Q fever [62]. Anti-phase II titers exceed the anti-phase I titers in sera from acute Q fever patients. However, in chronic Q fever patients, the anti-phase I titers exceed the titers directed against phase II *C. burnetii*. Patients with chronic Q fever endocarditis can also have high levels of serum IgA [60,62]. Although the duration of specific antibody titers against *C. burnetii* is over 5 years [63], without specific antigen stimulation antibody titers will eventually decline to negative levels even though the individual is immune. Immune individuals who are vaccinated run the risk of developing adverse reactions at the vaccination site.

Doxycycline is the treatment of choice for human acute Q fever [35]. Clarithromycin may also be a therapeutic option [64]. However, for treating chronic Q fever and especially endocarditis, drug combinations are needed and one of the most effective treatments is doxycycline plus hydroxychloroquine [65]. The substitution of ofloxacin for chloroquine might also be effective [35].

3. Modulation of immune responses

C. burnetii uses evasion and suppression of host immune responses to survive the hostile environment of the phagolysosome. As *C. burnetii* is susceptible to killing by active oxygen and nitrogen intermediates produced by host cells in response to infection [54], one survival strategy is to avoid host cell activation, allowing *C. burneii* to persist. Phase I *C. burnetii* does not activate human dendritic cells [66], as determined by IL-12p70 production or p38 mitogen-activated protein kinase phosphorylation [67]. Similarly, phase I *C. burnetii* LPS does not activate host macrophage antimicrobial responses via TLR4 [68]. Phase I LPS may mask TLR ligands from innate immune recognition, resulting in a lack of surveillance by dendritic cells and persistence of this microorganism [66]. In addition, lipoproteins and lipopeptides from phase I microorganisms do not activate macrophages by stimulating TLR2 [68], although TLR2 might play a role in cytokine production and granuloma formation after infection [69]. Therefore, by avoiding recognition by TLR, *Coxiella* can minimize detection by the host.

Particularly notable in cases of chronic Q fever, *C. burnetii* can also ensure its intracellular survival by suppressing host immune responses, allowing persistence of the microorganism. Peripheral blood cells from patients with chronic Q fever endocarditis did not proliferate when exposed *in vitro* to *C. burnetii* antigens [70], suggesting that the immune response of those patients was inadequate to eradicate the microorganism. This suppressive mechanism could involve the production of prostaglandin E2 and high levels of tumor necrosis factor, with consequently deleterious effects on the host's immune responses [18,71]. Patients with chronic Q fever also exhibited increased IL-10 secretion [72,73], leading to suppressed Th1-mediated cellular immunity.

Active suppression of host immune responses has been most thoroughly documented in the mouse Q fever model. The intraperitoneal injection of mice with 100 µg of killed phase I whole cell vaccine or viable microorganisms suppressed the lymphoproliferative responses of spleen cells to mitogens (concanavalin A, phytohemagglutinin, and pokeweed mitogen) [74]. Incorporation of radiolabeled thymidine was also suppressed when spleen cells from these animals were exposed to *C. burnetii* antigens in culture. Spleen cells from vaccinated mice were not simply unresponsive to stimulation by homologous antigens, they incorporated significantly less thymidine than cells cultured in the absence of antigen (i.e., stimulation index <1.0). Cultured spleen cells from naive mice proliferated in response to *C. burnetii* antigens. Therefore, the observed antigen-specific suppression after phase I antigen priming was not simply a failure to respond to antigen *in vitro*, but an event requiring prior sensitization by phase I *C. burnetii*. Suppression was not due to decreased viability of spleen cells or alteration of cellular kinetics in response to mitogens or antigen.

The components of phase I whole cells that caused immunosuppression were given the term "immunosuppressive complex (ISC)" [75]. Investigations into the nature of that complex revealed that the ISC could be inactivated and partitioned by chloroform-methanol (CM) (4:1) extraction. The suppressive components in either the residue, (CMR) or extract (CME) did not induce ISC activity in the host when given separately. LPS resides in the CMR after extraction, suggesting that phase I LPS is not the sole contributor to pathophysiological changes and immunosuppression in mice [76]. Reconstituting the CMR with CME (in CM) before injection into mice produced pathological reactions characteristic of phase I cells. The CMR suppressive component was sensitive to alkali, acid, periodate, lysozyme, and neuraminidase, but resistant to lipase and protease [75]. A suppressive component of CMR was attached to the cell matrix by disulphide bonds. Surprisingly, *C. burnetii* strains isolated from heart valves of immunosuppressed patients were not immunosuppressive in mice [77]. However, strains associated with acute Q fever were immunosuppressive [74].

Surprisingly, in light of its immunosuppressive capability is this microorganism's ability to also stimulate nonspecific immune responses resulting in tumor regression in guinea pigs and resistance to bacterial, protozoan, and viral infection [78–80].

4. Vaccines against Q fever

An efficacious Q fever vaccine was developed and available for human vaccination only a few years after discovery of the etiologic agent. This preparation was rather crude, consisting of formalin-killed and ether-extracted *C. burnetii* containing 10% yolk sac, but it was effective in protecting human volunteers from disease after aerosol challenge [81]. In those early studies, the antigenic nature of the vaccine was not known. More recent vaccines were prepared from phase I microorganisms, as those preparations were 100–300 times more potent than phase II vaccines [82]. Improved purification methods were eventually developed to exclude egg proteins and lipids. Vaccine efficacy of these more highly purified preparations was also demonstrated in human volunteers [83]. However, although efficacious, the use of this and other early phase I cellular vaccines was occasionally accompanied by adverse reactions at the vaccination site, including induration or the formation of sterile abscesses or granulomas [84]. People with a history of Q fever or those previously vaccinated were at risk for developing these adverse reactions [84]. Approximately 3% of persons vaccinated for the ninth and tenth time developed severe persistent reactions [85]. Screening for prior immunity with a skin test that prevented immune individuals from being vaccinated resulted in a dramatic decrease in the incidence of adverse reactions after vaccination [86]. Currently, skin testing is used to assess the potential for developing adverse vaccination reactions, although some laboratories also measure the level of specific antibodies against *C. burnetii* [87]. Cellular *C. burnetii* vaccines currently in use are safe and efficacious if the recipients are not immune due to prior *C. burnetii* infection.

The most thoroughly tested Q fever vaccine in use today is "Q-Vax." This formalin-killed, phase I cellular vaccine is produced and licensed for use in Australia [87]. In Australian studies, this vaccine was 100% effective in preventing clinical Q fever in occupationally at-risk individuals [87]. The duration of protection was over 5 years. However, this vaccine cannot be administered without prior determination of immunity and exclusion of those testing positive. A similar product, while not licensed, is administered as an Investigational New Drug in the US and is used to vaccinate at-risk persons at the US Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD. Many countries do not have an approved vaccine for Q fever. The remarkable efficacy of Q-Vax (a vaccine manufactured using the phase I Henzerling strain originally isolated in Italy) in Australian trials suggested that there is cross-protection between *C. burnetii* variants. Furthermore, that lymphoproliferative responses of individuals vaccinated with Q-Vax were

equivalent when peripheral blood cells were cultured in the presence of Priscilla strain, Henzerling strain, or phase I Nine Mile strain *C. burnetii* antigens [88] lends support to this hypothesis.

Although attenuated microorganisms generally are not used as Q fever vaccines, a phase II attenuated strain, designated M-44, was developed from the Greek 'Grita' strain in the former Soviet Union [89]. This vaccine was capable of causing adverse reactions in humans and caused myocarditis, hepatitis, liver necrosis, granuloma formation, and splenitis in guinea pigs [90]. Human vaccinees did not develop anti-phase I antibodies and anti-phase II levels were variable and at low titer.

Although the use of cellular Q fever vaccines mandates testing for prior immunity, there are potential difficulties that could be encountered. Using serological titers as an indicator of immunity might not eliminate the risk of adverse vaccination reactions as specific antibody titers decrease after acute infection [63] and may not accurately reflect the immune status of the individual. Skin tests are time consuming, costly, and would not give accurate results if incorrectly applied or misinterpreted. Therefore, laboratories are attempting to develop Q fever vaccines that will pose a lesser risk if given to someone with preexisting immunity. Q fever vaccines that do not require screening for prior immunity could reduce costs and time associated with vaccination.

One candidate vaccine was prepared by extracting phase I whole cells with a mixture of chloroform and methanol [91]. CMR vaccine (introduced previously) did not cause adverse reactions in mice at doses higher than those found associated with severe adverse reactions after vaccination with phase I cellular vaccine [91]. Phase I human trials showed that the CMR vaccine could be safely administered to human volunteers unscreened for prior immunity [92]. Efficacy of the CMR Q fever vaccine has been demonstrated in laboratory rodents, sheep, and non-human primates [76,93–95].

Other vaccine candidates have been tested. A fusion protein, consisting of *C. burnetii* outer membrane protein 1 (P1) and heat-shock protein B (HspB) demonstrated some protective efficacy [96]. However, a mixture of eight recombinant *C. burnetii* proteins that were expressed in *E. coli* from potential virulence genes (Omp, Pmm, HspB, Fbp, Orf 410, Crc, CbMip, and MucZ) were found to be antigenic (except Pmm) but not protective when BALB/c mice were challenged intraperitoneally with the phase I Nine Mile strain [97]. Vaccination with single recombinant *C. burnetii* proteins (Rcom, rP1, rCbMip, or rP28) also was not protective [98].

The ultimate goal of current vaccine efforts is to develop an efficacious vaccine that can be safely administered to individuals at risk of infection, does not require screening for prior immunity, and can be licensed. Cellular Q fever vaccines probably would not be licensable in the U.S. due to safety concerns. A licensed Q fever vaccine would benefit those occupationally at risk for Q fever, those residing in areas endemic for Q fever, and soldiers or civilians who may be exposed as the result of a bioterrorism or biowarfare attack. Important questions remaining to be decided include (1) how does the microorganism's ability to persist in the host, cause adverse outcomes in pregnancy, and modulate host immune responses impact vaccination strategy; and (2) since epidemiological surveys suggest that approximately one-seventh of the population tested have been exposed to *C. burnetii*, who should be vaccinated?

5. Perspective

Few microorganisms are as fascinating as this obligate intracellular pathogen that requires the inhospitable (for most bacteria) eukaryotic phagolysosome for growth. Immunomodulatory capabilities range from immunosuppression seen in chronic Q fever patients to nonspecific stimulation after injection of vaccine that is able to regress tumors and allow mice to nonspecifically resist infection. *C. burnetii* is also a significant biological warfare agent and during the Cold War was weaponized by the Soviets and US [99]. Classification as a Category B biothreat agent, due to the agent's high infectivity at low aerosol exposure doses, great stability of the infectious particles, and significant disease morbidity, has stimulated investigations into improved Q fever vaccines, diagnostics, and therapy and highlighted the need for greater understanding of host–pathogen interactions. The pace of research into improved Q fever vaccines that can be administered without assessment of prior immunity needs to be accelerated. While the efficacy of cellular Q fever vaccines is exceptional, these vaccines are not widely available and probably will not be licensed (in the U.S.). Promising subcellular or recombinant Q fever vaccines are proving to be elusive. However, a vaccine cocktail of appropriate recombinant and/or purified subcellular components administered with an appropriate adjuvant or targeted to the appropriate site could be the key to an efficacious, safe Q fever vaccine. Finally, the interplay between the positive and negative immunomodulatory capabilities of this microorganism needs to be better understood.

Acknowledgments

References

[1] Derrick EH. Q fever, a new fever entity: clinical features, diagnosis and laboratory investigation. Med J Aust 1937;2:281.

[2] Davis G, Cox H. A filter-passing infectious agent isolated from ticks. I. Isolation from *Dermacentor andersoni*, reactions in animals, and filtration experiments. Public Health Rep 1938;53:2259–67.

[3] Cox H. A filter-passing infectious agent isolated from ticks. III. Description of organism and cultivation experiments. Public Health Rep 1938;53:2270–6.

[4] Dyer RE. A filter-passing infectious agent isolated from ticks. IV. Human infection. Public Health Rep 1938;53:2277–83.

[5] Cox HR. Studies of a filter-passing infectious agent isolated from ticks. V. Further attempts to cultivate in cell-free media. Suggested classification. Public Health Rep 1939;54:1822–7.

[6] Derrick EH. *Rickettsia burnetii*: the cause of "Q" fever. Med J Aust 1939;1:14.

[7] Philip CB. Comments on the name of the Q fever organism. Public Health Rep 1948;63:58.

[8] Luoto L, Huebner RJ. Q fever studies in Southern California. IX. Isolation of Q fever organisms from paturient placentas of naturally infected dairy cows. Public Health Rep 1950;65:541.

[9] Welsh HH, Lennette EH, Abinanti FR, Winn JF. Q fever in California. IV. Occurrence of *Coxiella burnetii* in the placenta of naturally infected sheep. Public Health Rep 1951;66(45):1473–7.

[10] Waag D, Williams J, Peacock M, Raoult D. Methods for isolation, amplification, and purification of *Coxiella burnetii*. In: Williams J, Thompson HA, editors. Q fever: the biology of *Coxiella burnetii*. Boca Raton: CRC Press; 1991. p. 73–115.

[11] McCaul TF, Williams JC. Developmental cycle of *Coxiella burnetii*: structure and morphogenesis of vegetative and sporogenic differentiations. J Bacteriol 1981;147(3):1063–76.

[12] Samuel JE. Developmental cycle of *Coxiella burnetii*. In: Brun YV, Shimkets LJ, editors. Procaryotic development. Washington, DC: ASM Press; 2000. p. 427–40.

[13] McCaul TF, Banerjee-Bhatnagar N, Williams JC. Antigenic differences between *Coxiella burnetii* cells revealed by postembedding immunoelectron microscopy and immunoblotting. Infect Immun 1991;59(9):3243–53.

[14] Stoker MG, Fiset P. Phase variation of the Nine Mile and other strains of *Rickettsia burneti*. Can J Microbiol 1956;2(3):310–21.

[15] Moos A, Hackstadt T. Comparative virulence of intra- and interstrain lipopolysaccharide variants of *Coxiella burnetii* in the guinea pig model. Infect Immun 1987;55(5):1144–50.

[16] Hoover TA, Culp DW, Vodkin MH, Williams JC, Thompson HA. Chromosomal DNA deletions explain phenotypic characteristics of two antigenic variants, phase II and RSA 514 (crazy), of the *Coxiella burnetii* nine mile strain. Infect Immun 2002;70(12):6726–33.

[17] Vodkin MH, Williams JC. Overlapping deletion in two spontaneous phase variants of *Coxiella burnetii*. J Gen Microbiol 1986;132(Pt 9):2587–94.

[18] Mege JL, Maurin M, Capo C, Raoult D. *Coxiella burnetii*: the 'query' fever bacterium. A model of immune subversion by a strictly intracellular microorganism. FEMS Microbiol Rev 1997;19(4):209–17.

[19] Capo C, Lindberg FP, Meconi S, Zaffran Y, Tardei G, Brown EJ, et al. Subversion of monocyte functions by *Coxiella burnetii*: impairment of the cross-talk between alphavbeta3 integrin and CR3. J Immunol 1999;163(11):6078–85.

[20] Honstettre A, Ghigo E, Moynault A, Capo C, Toman R, Akira S, et al. Lipopolysaccharide from *Coxiella burnetii* is involved in bacterial phagocytosis, filamentous actin reorganization, and inflammatory responses through Toll-like receptor 4. J Immunol 2004;172(6):3695–703.

[21] Akporiaye ET, Baca OG. Superoxide anion production and superoxide dismutase and catalase activities in *Coxiella burnetii*. J Bacteriol 1983;154(1):520–3.

[22] Mo YY, Cianciotto NP, Mallavia LP. Molecular cloning of a *Coxiella burnetii* gene encoding a macrophage infectivity potentiator (Mip) analogue. Microbiology 1995;141(Pt 11):2861–71.

[23] Zamboni DS, McGrath S, Rabinovitch M, Roy CR. *Coxiella burnetii* express type IV secretion system proteins that function similarly to components of the *Legionella pneumophila* Dot/Icm system. Mol Microbiol 2003;49(4):965–76.

[24] Heinzen RA, Frazier ME, Mallavia LP. *Coxiella burnetii* superoxide dismutase gene: cloning, sequencing, and expression in *Escherichia coli*. Infect Immun 1992;60(9):3814–23.

[25] DeLay PD, Lennette EH, Deome KB. Q fever in California. II. Recovery of *Coxiella burnetii* from naturally infected airborne dust. J Immunol 1959;65:211–20.

[26] Tigertt WD, Benenson AS, Gouchenour WS. Airborne Q fever. Bacteriol Rev 1961;25:285–93.

[27] Behymer DE, Biberstein EL, Riemann HP, Franti CE, Sawyer M, Ruppanner R, et al. Q fever (*Coxiella burnetii*) investigations in dairy cattle: challenge of immunity after vaccination. Am J Vet Res 1976;37(6):631–4.

[28] Rose M, Wemheuer W, Schmidt FW. Follow-up studies of the detection of IgG1 antibodies to *Coxiella burnetii* in blood and milk from cattle in relation to the reproductive cycle and milk production. Dtsch Tierarztl Wochenschr 1994;101(12):484–6.

[29] Langley JM, Marrie TJ, Leblanc JC, Almudevar A, Resch L, Raoult D. *Coxiella burnetii* seropositivity in parturient women is associated with adverse pregnancy outcomes. Am J Obstet Gynecol 2003;189(1):228–32.

[30] Raoult D, Fenollar F, Stein A. Q fever during pregnancy: diagnosis, treatment, and follow-up. Arch Intern Med 2002;162(6): 701–4.

[31] Marrie T. Epidemiology of Q fever. In: Marrie T, editor. Q fever: the disease. Boca Raton: CRC Press; 1990. p. 49–70.

[32] Russell-Lodrigue KE, Zhang GQ, McMurray DN, Samuel JE. Clinical and pathologic changes in a guinea pig aerosol challenge model of acute Q fever. Infect Immun 2006;74(11):6085–91.

[33] Hackstadt T, Williams JC. Biochemical stratagem for obligate parasitism of eukaryotic cells by *Coxiella burnetii*. Proc Natl Acad Sci U S A 1981;78(5):3240–4.

[34] Khavkin TN. Pathologo-anatomic and experimental study of the morphology of Q-fever. Arkh Patol 1977;39(2):75–84.

[35] Maurin M, Raoult D. Q fever. Clin Microbiol Rev 1999;12(4):518–53.

[36] Sawyer LA, Fishbein DB, McDade JE. Q fever: current concepts. Rev Infect Dis 1987;9(5):935–46.

[37] Tigertt WD, Benenson AS. Studies on Q fever in man. Trans Assoc Am Phys 1956;69:98–104.

[38] Derrick EH. The course of infection with *Coxiella burneti*. Med J Aust 1973;1(21):1051–7.

[39] Marrie T. Acute Q Fever. In: Marrie T, editor. Q fever, the disease. Boca Raton: CRC Press; 1990. p. 125–60.

[40] Fournier PE, Marrie TJ, Raoult D. Diagnosis of Q fever. J Clin Microbiol 1998;36(7):1823–34.

[41] Ferrante MA, Dolan MJ. Q fever meningoencephalitis in a soldier returning from the Persian Gulf War. Clin Infect Dis 1993;16(4):489–96.

[42] Tissot-Dupont H, Vaillant V, Rey S, Raoult D. Role of sex, age, previous valve lesion, and pregnancy in the clinical expression and outcome of Q fever after a large outbreak. Clin Infect Dis 2007;44(2): 232–7.

[43] Raoult D, Raza A, Marrie T. Q fever endocarditis and other forms of chronic Q fever. In: Marrie T, editor. Q fever: the disease. Boca Raton: CRC Press; 1990. p. 179–99.

[44] Heard SR, Ronalds CJ, Heath RB. *Coxiella burnetii* infection in immunocompromised patients. J Infect 1985;11(1):15–8.

[45] Raoult D, Levy PY, Dupont HT, Chicheportiche C, Tamalet C, Gastaut JA, et al. Q fever and HIV infection. AIDS 1993;7(1):81–6.

[46] Tobin MJ, Cahill N, Gearty G, Maurer B, Blake S, Daly K, et al. Q fever endocarditis. Am J Med 1982;72(3):396–400.

[47] Brouqui P, Dupont HT, Drancourt M, Berland Y, Etienne J, Leport C, et al. Chronic Q fever. Ninety-two cases from France, including 27 cases without endocarditis. Arch Intern Med 1993;153(5):642–8.

[48] Fenollar F, Fournier PE, Carrieri MP, Habib G, Messana T, Raoult D. Risks factors and prevention of Q fever endocarditis. Clin Infect Dis 2001;33(3):312–6.

[49] Marmion BP, Storm PA, Ayres JG, Semendric L, Mathews L, Winslow W, et al. Long-term persistence of *Coxiella burnetii* after acute primary Q fever. QJM 2005;98(1):7–20.

[50] Penttila IA, Harris RJ, Storm P, Haynes D, Worswick DA, Marmion BP. Cytokine dysregulation in the post-Q-fever fatigue syndrome. QJM 1998;91(8):549–60.

[51] Helbig K, Harris R, Ayres J, Dunckley H, Lloyd A, Robson J, et al. Immune response genes in the post-Q-fever fatigue syndrome, Q fever endocarditis and uncomplicated acute primary Q fever. QJM 2005;98(8):565–74.

[52] Zhang G, To H, Russell KE, Hendrix LR, Yamaguchi T, Fukushi H, et al. Identification and characterization of an immunodominant 28-kilodalton *Coxiella burnetii* outer membrane protein specific to isolates associated with acute disease. Infect Immun 2005;73(3):1561–7.

[53] Glazunova O, Roux V, Freylikman O, Sekeyova Z, Fournous G, Tyczka J, et al. *Coxiella burnetii* genotyping. Emerg Infect Dis 2005;11(8):1211–7.

[54] Brennan RE, Russell K, Zhang G, Samuel JE. Both inducible nitric oxide synthase and NADPH oxidase contribute to the control of virulent phase I *Coxiella burnetii* infections. Infect Immun 2004;72(11):6666–75.

[55] Kishimoto RA, Rozmiarek H, Larson EW. Experimental Q fever infection in congenitally athymic nude mice. Infect Immun 1978;22(1):69–71.

[56] Turco J, Thompson HA, Winkler HH. Interferon-gamma inhibits growth of *Coxiella burnetii* in mouse fibroblasts. Infect Immun 1984;45(3):781–3.

[57] Humphres RC, Hinrichs DJ. Role of antibody in *Coxiella burnetii* infection. Infect Immun 1981;31(2):641–5.

[58] Kishimoto RA, Walker JS. Interaction between *Coxiella burnetii* and guinea pig peritoneal macrophages. Infect Immun 1976;14(2):416–21.

[59] Honstettre A, Meghari S, Nunes JA, Lepidi H, Raoult D, Olive D, et al. Role for the CD28 molecule in the control of *Coxiella burnetii* infection. Infect Immun 2006;74(3):1800–8.

[60] Dupuis G, Peter O, Luthy R, Nicolet J, Peacock M, Burgdorfer W. Serological diagnosis of Q fever endocarditis. Eur Heart J 1986;7(12):1062–6.

[61] Peter O, Dupuis G, Burgdorfer W, Peacock M. Evaluation of the complement fixation and indirect immunofluorescence tests in the early diagnosis of primary Q fever. Eur J Clin Microbiol 1985;4(4):394–6.

[62] Peacock MG, Philip RN, Williams JC, Faulkner RS. Serological evaluation of O fever in humans: enhanced phase I titers of immunoglobulins G and A are diagnostic for Q fever endocarditis. Infect Immun 1983;41(3):1089–98.

[63] Waag D, Chulay J, Marrie T, England M, Williams J. Validation of an enzyme immunoassay for serodiagnosis of acute Q fever. Eur J Clin Microbiol Infect Dis 1995;14(5):421–7.

[64] Gikas A, Kofteridis DP, Manios A, Pediaditis J, Tselentis Y. Newer macrolides as empiric treatment for acute Q fever infection. Antimicrob Agents Chemother 2001;45(12):3644–6.

[65] Raoult D, Houpikian P, Tissot Dupont H, Riss JM, Arditi-Djiane J, Brouqui P. Treatment of Q fever endocarditis: comparison of 2 regimens containing doxycycline and ofloxacin or hydroxychloroquine. Arch Intern Med 1999;159(2):167–73.

[66] Shannon JG, Howe D, Heinzen RA. Virulent *Coxiella burnetii* does not activate human dendritic cells: role of lipopolysaccharide as a shielding molecule. Proc Natl Acad Sci USA 2005;102(24):8722–7.

[67] Shannon JG, Howe D, Heinzen RA. Lack of dendritic cell maturation following infection by *Coxiella burnetii* synthesizing different lipopolysaccharide chemotypes. Ann N Y Acad Sci 2005;1063:154–60.

[68] Zamboni DS, Campos MA, Torrecilhas AC, Kiss K, Samuel JE, Golenbock DT, et al. Stimulation of toll-like receptor 2 by *Coxiella burnetii* is required for macrophage production of pro-inflammatory cytokines and resistance to infection. J Biol Chem 2004;279(52):54405–15.

[69] Meghari S, Honstettre A, Lepidi H, Ryffel B, Raoult D, Mege JL. TLR2 is necessary to inflammatory response in *Coxiella burnetii* infection. Ann N Y Acad Sci 2005;1063:161–6.

[70] Koster FT, Williams JC, Goodwin JS. Cellular immunity in Q fever: specific lymphocyte unresponsiveness in Q fever endocarditis. J Infect Dis 1985;152(6):1283–9.

[71] Koster FT, Williams JC, Goodwin JS. Cellular immunity in Q fever: modulation of responsiveness by a suppressor T cell-monocyte circuit. J Immunol 1985;135(2):1067–72.

[72] Ghigo E, Capo C, Raoult D, Mege JL. Interleukin-10 stimulates *Coxiella burnetii* replication in human monocytes through tumor necrosis factor down-modulation: role in microbicidal defect of Q fever. Infect Immun 2001;69(4):2345–52.

[73] Capo C, Zaffran Y, Zugun F, Houpikian P, Raoult D, Mege JL. Production of interleukin-10 and transforming growth factor beta by peripheral blood mononuclear cells in Q fever endocarditis. Infect Immun 1996;64(10):4143–7.

[74] Damrow TA, Williams JC, Waag DM. Suppression of in vitro lymphocyte proliferation in C57BL/10 ScN mice vaccinated with phase I *Coxiella burnetii*. Infect Immun 1985;47(1):149–56.

[75] Waag DM, Williams JC. Immune modulation by *Coxiella burnetii*: characterization of a phase I immunosuppressive complex differentially expressed among strains. Immunopharmacol Immunotoxicol 1988;10(2):231–60.

[76] Williams JC, Damrow TA, Waag DM, Amano K. Characterization of a phase I *Coxiella burnetii* chloroform-methanol residue vaccine that induces active immunity against Q fever in C57BL/10 ScN mice. Infect Immun 1986;51(3):851–8.

[77] Williams JC. Infectivity, virulence, and pathogenesis of *Coxiella burnetii* for various hosts. In: Williams JC, Thompson HA, editors. Q fever: the biology of *Coxiella burnetii*. Boca Raton, FL: CRC Press, Inc.; 1991. p. 21–71.

[78] Clark IA. Resistance to *Babesia* spp. and *Plasmodium* sp. in mice pretreated with an extract of *Coxiella burnetii*. Infect Immun 1979;24(2):319–25.

[79] Kelly MT, Granger DL, Ribi E, Milner KC, Strain SM, Stoenner HG. Tumor regression with Q fever rickettsiae and a mycobacterial glycolipid. Cancer Immunol Innunother 1976;1:187–91.

[80] Waag DM, Kende M, Damrow TA, Wood OL, Williams JC. Injection of inactivated phase I *Coxiella burnetii* increases non-specific resistance to infection and stimulates lymphokine production in mice. Ann N Y Acad Sci 1990;590:203–14.

[81] Smadel JE, Snyder MJ, Robins FC. Vaccination against Q fever. Am J Hyg 1948;47:71–8.

[82] Ormsbee RA, Bell EJ, Lackman DB, Tallent G. The influence of phase on the protective potency of Q fever vaccine. J Immunol 1964;92:404–12.

[83] Fiset P. Vaccination against Q fever. In: Vaccines against viral and rickettsial diseases in man, PAHO Science Publication Number 147. Wahington, DC: Pan American Health Organization; 1966. p. 528–31.

[84] Bell JF, Lackman DB, Meis A, Hadlow WJ. Recurrent reaction of site of Q fever vaccination in a sensitized person. Mil Med 1964;129:591–5.

[85] Benenson AS. Q fever vaccine: efficacy and present status. In: Smadel JE, editor. Symposium on Q fever. Washington, DC: Walter Reed Army Institute of Medical Science, Publ. No. 6, Government Printing Office; 1959.

[86] Lackman D, Bell EJ, Bell JF, Pickens E. Intradermal sensitivity testing in man with a purified vaccine for Q fever. Am J Public Health 1962;52:87–93.

[87] Ackland JR, Worswick DA, Marmion BP. Vaccine prophylaxis of Q fever. A follow-up study of the efficacy of Q-Vax (CSL) 1985–1990. Med J Aust 1994;160(11):704–8.

[88] Izzo AA, Marmion BP, Hackstadt T. Analysis of the cells involved in the lymphoproliferative response to *Coxiella burnetii* antigens. Clin Exp Immunol 1991;85(1):98–108.

[89] Genig VA. A live vaccine 1/M-44 against Q fever for oral use. J Hyg Epidemiol Microbiol Immunol 1968;12:265–73.

[90] Johnson JW, McLeod CG, Stookey JL, Higbee GA, Pedersen Jr CE. Lesions in guinea pigs infected with *Coxiella burnetii* strain M-44. J Infect Dis 1977;135(6):995–8.

[91] Williams JC, Cantrell JL. Biological and immunological properties of *Coxiella burnetii* vaccines in C57BL/10ScN endotoxin-nonresponder mice. Infect Immun 1982;35(3):1091–102.

[92] Fries LF, Waag DM, Williams JC. Safety and immunogenicity in human volunteers of a chloroform-methanol residue vaccine for Q fever. Infect Immun 1993;61(4):1251–8.

[93] Brooks DL, Ermel RW, Franti CE, Ruppanner R, Behymer DE, Williams JC, et al. Q fever vaccination of sheep: challenge of immunity in ewes. Am J Vet Res 1986;47(6):1235–8.

[94] Waag DM, England MJ, Pitt ML. Comparative efficacy of a *Coxiella burnetii* chloroform:methanol residue (CMR) vaccine and a licensed cellular vaccine (Q-Vax) in rodents challenged by aerosol. Vaccine 1997;15(16):1779–83.

[95] Waag DM, England MJ, Tammariello RF, Byrne WR, Gibbs P, Banfield CM, et al. Comparative efficacy and immunogenicity of Q fever chloroform:methanol residue (CMR) and phase I cellular (Q-Vax) vaccines in cynomolgus monkeys challenged by aerosol. Vaccine 2002;20(19–20):2623–34.

[96] Li Q, Niu D, Wen B, Chen M, Qiu L, Zhang J. Protective immunity against Q fever induced with a recombinant P1 antigen fused with HspB of *Coxiella burnetii*. Ann N Y Acad Sci 2005;1063:130–42.

[97] Tyczka J, Eberling S, Baljer G. Immunization experiments with recombinant *Coxiella burnetii* proteins in a murine infection model. Ann N Y Acad Sci 2005;1063:143–8.

[98] Zhang GQ, Samuel JE. Identification and cloning potentially protective antigens of *Coxiella burnetii* using sera from mice experimentally infected with Nine Mile phase I. Ann N Y Acad Sci 2003;990:510–20.

[99] Alibek K, Biohazard. The chilling true story of the largest covert biological weapons program in the world. New York: Random House; 1999.